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## **The influence of neonatal environment on piglet play behaviour and post-weaning social and cognitive development**

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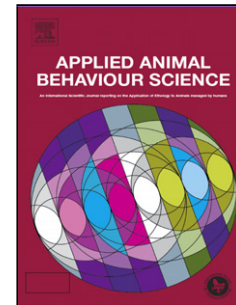
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The influence of neonatal environment on piglet play behaviour and post-weaning social and cognitive development.

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Highlights:

- Compared piglet play behaviour in two neonatal environments pre- and post-weaning.
- Play behaviour is greater in piglets housed in complex and enriched environments.
- More playful piglets perform better in Spontaneous Object Recognition Tests.
- Play is dependent on present environmental stimulus.
- Direct and indirect effects on welfare e.g. reduced chronic aggression and stress.

21

22 Abstract

23 Research has shown that the domestic pig is highly playful throughout its development and that play is  
 24 an important aspect of social and cognitive development. Therefore the neonatal environment is  
 25 fundamental to successful stimulation of play in neonatal pigs, which could have indirect and direct  
 26 socio-cognitive effects on pigs post-weaning and therefore influence social interactions known to cause  
 27 welfare concerns (e.g. aggression during mixing). This study investigated how play pre- and post-  
 28 weaning developed in two neonatal environments (NE); the conventional farrowing crate (NEC) and a  
 29 more environmentally complex alternative PigSAFE pen (NEP) and to discover whether this had an effect  
 30 on piglet' cognitive abilities in Spontaneous Object Recognition Tests for two retention times (15 and 60  
 31 minutes) post-weaning. Hourly focal sampling was used to record play behaviours pre- and post-  
 32 weaning in 72 piglets of mixed sex (36 per NE) from a total population of 117 piglets from 12 litters. Out  
 33 of the 72 piglets, 24 were used in the cognitive Spontaneous Object Recognition tests five weeks post-  
 34 weaning. Linear mixed models showed that NEP piglets displayed play behaviours quicker after birth  
 35 than NEC piglets: locomotor ( $F = 7.62_{(1,11)}$ ,  $P = 0.020$ ); sow interaction ( $F = 5.27_{(1,11)}$ ,  $P = 0.045$ ); and social  
 36 interaction ( $F = 23.61_{(1,11)}$ ,  $P < 0.001$ ). NEP piglets played more pre-weaning than NEC piglets ( $F =$   
 37  $5.06_{(1,71)}$ ,  $P = 0.051$ ) and despite initial higher levels of aggression at weaning, displayed less chronic  
 38 aggression post-weaning as indicated by lesion scores of all piglets ( $F = 27.05_{(1,116)}$ ,  $P < 0.001$ ). NE was  
 39 shown to have a significant effect on the 15 minute cognitive retention test; with NEP piglets spending  
 40 more time interacting with the novel object than the familiar, compared to NEC piglets ( $F = 5.39_{(1,23)}$ ,  $P =$   
 41  $0.045$ ). There was no NE effect for the 60 minute retention test. It was concluded that play is  
 42 fundamental to successful socio-cognitive development (e.g. aggressive conflicts) and relates to play  
 43 function theories of training for the unexpected. Its effect on play behaviours are short-term and highly

dependent on present environmental stimulus, suggesting that any long-term benefits play may have on an animal's welfare can only be achieved by regular stimulation throughout its life (e.g. constant enrichment).

Keywords: Play, alternative housing, farrowing, enrichment

## 1. Introduction

Research into play behaviour has shown it to be fundamental for the physical, physiological and psychological development of mammals, particularly the development of cognitive and social abilities (Fagen, 1981; Špinka et al., 2001). As a result play behaviour (or the lack thereof) has been used as a welfare 'iceberg indicator' to highlight concerns for captive animals, e.g. boredom (Held and Špinka, 2011; Dybkjaer, 1992). Mammalian play is a cognitively demanding activity and is concentrated during neonatal development (Špinka et al., 2001).

The domestic pig (*Sus scrofa domestica*) is a social, intelligent mammal (D'Eath and Turner, 2009; Gieling et al., 2011). The pig's commercial environment, whether indoor or outdoor, imposes physical and behavioural restrictions, and subjects it to several stressful events during its production life (e.g. weaning) (Marchant-Forde, 2009). Several studies on pigs, involving object recognition, spatial memory and problem-solving, have demonstrated a high level of cognitive skill (Gielsing et al., 2011; Moustgaard et al., 2002). Research also shows that pig play behaviour extends across all play behaviour categories; locomotor, social and object, and that it has sex and age dependent aspects (Donaldson et al., 2002; Newberry et al., 1988).

Interactions between sow and piglet within the neonatal environment (NE) are critical for piglet survival (English and Smith, 1975; Marchant et al., 2000), but also for socio-cognitive development. Research indicates that several factors are influenced by the NE, including sow/piglet behaviour (e.g. Bolhuis et al., 2005, 2006; Cronin et al., 1996; De Jonge et al., 1996; Melotti et al., 2011; Olsson et al., 1999; Siegford et al., 2008). Lack of understanding on how influential these effects on piglet development are may mask just how important the NE is. Research has shown that restriction in this environment can disturb development of social skills and stress coping mechanisms, resulting in higher stress and aggression levels in adult pigs (e.g. Peterson et al., 2005). This supports the theory that play acts as training for the future (e.g. responses to novelty and social interactions; including aggressive conflicts) (Špinka et al., 2001).

The aims of this study were to investigate whether piglets reared in conventional farrowing crates (NEC) or more environmentally complex alternative farrowing pens (PigSAFE pen - NEP) show different play behaviour and development pre- and post-weaning, and whether this results in variation of cognitive abilities post-weaning. It was hypothesised that if play behaviour is key to successful socio-cognitive development of neonates, then the NE must have an indirect significant impact, as play behaviour can be stimulated or restricted by the environment. Therefore, piglets reared in a more complex environment should show greater socio-cognitive development than piglets reared in a standard commercial NE.

## 2. Material and methods

### 2.1 Animals and Housing

Data were collected at the SRUC Pig Unit (Midlothian, Scotland) between March and June 2011. A total of 117 piglets, bred from commercial cross-bred dams (Large White x Landrace) and sired by Pietrain boars were used. Of the 117, 57 were born in the first NE, the standard farrowing crate (NEC), and 60 were born in the second NE, the PigSAFE pen (NEP). Piglets were produced from 12 sows, with six sows per NE of equally varying parity. Litter size was not equalised and was dependent on natural biological variation. However cross-fostering was permitted as per normal husbandry routines to improve piglet survival. This was done within NE. The pig unit was managed on a batch system, involving a group of sows farrowing simultaneously at three week intervals. As a result of all-in-all-out management, farrowing system type was alternating, so comparisons of the NEs could not be run simultaneously.

The standard farrowing crate (NEC) was used to represent a barren environment as it is a restrictive environment to both sow and piglets in terms of physical movement and mental stimulation (Figure 1a). The crate had a solid concrete floor, apart from a small dunging area to the rear of the sow (0.5 x 0.5 m<sup>2</sup>). Natural light was provided by windows in the farrowing house. In addition artificial lighting was on between 0700-1600 daily, with permanent lighting on in the creep area. Both the sow and her piglets were physically isolated from other pigs. Two handfuls of long-stemmed straw were given daily, which both the sow and piglets had access to, as this was standard farm practise for the NEC. The sow is restricted to the central area via parallel bars, and her piglets are able to move around her and have access to a heated and lit creep area at the front of the crate.

The newly developed Piglet and Sow Alternative Farrowing Environment, or PigSAFE (NEP), was developed based on the design criteria proposed by Baxter et al. (2011) and described in Edwards et al. (2012). Flooring was solid, insulated concrete with a slatted dunging area (Triband metal slats, 9mm

void). Lighting was provided artificially between 0700-1600 daily, with night lights remaining on at a lower lux. Sows were provided with 2kg long-stemmed straw pre-farrowing which was replenished daily if needed. Approximately 24h post-farrowing straw was removed if dirty and two handfuls of additional long-stemmed straw was provided daily until weaning. NEP provides visual and some physical interaction with neighbouring sows and piglets through the barred windows, and also has sloped walls, which protect piglets from crushing and, inadvertently, add complexity to the environment (Figure 1b).

Piglets were introduced to solid feed (Compound pellet creep feed, Scotlean Pigs Ltd., Primary Diets – AB Abri Ltd., Yorks, UK) one week before weaning by floor scattering pellets within the creep area. Weaning occurred at 27 days old, during which piglets were removed from sows and underwent several management procedures (e.g. vaccination and ear tagging) before being moved to weaner pens. Weaner pens were 3 x 6 m<sup>2</sup>, with solid concrete floors and solid walls (1.5 m high) and deep-straw bedding. Pens were mucked out and long-stemmed straw for bedding was replenished daily (4-5 kg as required). Lighting regime was 10hrs of artificial light (07:00 – 17:00). Room temperature and ventilation were mechanically controlled; room temperature was set at 25-27°C for the first few days, dropping to 21°C after one week. Piglets had ad libitum access to suitable feed and water. Handling of piglets was performed as calmly and swiftly as possible to minimise stress. Approximately 20 piglets were housed per pen, with two litters from the same NE being mixed to make a group (three pens per NE). Weaner group sizes: NEC - pen A = 17, pen B = 20, and pen C = 20; NEP - pen D = 20, pen E = 20, and pen F = 20. Efforts were made to mix litters of similar size to minimise bullying (Francis et al., 1996).

This project was reviewed and approved by SRUC's ethical review committee and all routine animal management procedures were adhered to by trained staff.



134

## 135 2.2 Experimental Design

136 The experimental study was split into three phases in order to address the hypothesis. Phase 1 (piglets  
137  $\leq 27$  days old) and Phase 2 (piglets aged  $28 \leq 56$  days) involved investigating play behaviours pre- and  
138 post-weaning, while Phase 3 involved the application of Spontaneous Object Recognition Tests to piglets  
139 from both NEs post-weaning (approximately 56-70 days old).

140

141 A behavioural ethogram (Table 1) was developed and tested in pilot footage of digitally video recorded  
142 piglets from farrowing to two weeks old in NECs from 2009. Play behaviours had been verified by  
143 previous research (e.g. Blackshaw et al., 1997, Bolhuis et al., 2005, Chaloupková et al., 2007, Donaldson  
144 et al., 2002, Jensen et al., 2001, Newberry et al., 1988). As a result of this research, 'play fighting'  
145 behaviours (e.g. biting) were excluded from the current study.

146

## 147 2.3 Phase 1: Comparisons of Pre-weaning Play Behaviours

148 All litters from each NE treatment were digitally video recorded (Low-lux B/W waterproof cameras: SK-  
149 2020XC/SO, RF Concepts Ltd, Belfast, Ireland and Geovision GV-DVR, ezCCTV Ltd, Herts, UK)  
150 continuously for four days post-farrowing. In order not to disrupt managerial procedures or maternal  
151 behaviour, piglet handling was minimal during this period. Data were collected on the latency post-  
152 farrowing for the first locomotor play behaviours to be performed by any piglet, within the four days for  
153 each litter. At such an early age and using only video footage it was too difficult to distinguish definite

social play interactions and therefore latency for first sow-piglet interaction and other social physical interactions (e.g. nudge – Table 1) were recorded but not specifically defined as social play.

Following the initial four days, using digitally recorded video footage, each litter underwent focal sampling (Martin and Bateson, 2007) hourly for three minutes from 08:00 to 16:00 on Mondays, Thursdays and Sundays up until weaning (4 – 27 days of age). Between 08:05 and 08:55 every day, piglets were picked up daily and individually labelled with a number on their backs in black permanent marker (Sharpie® Magnum chisel tip). The same markers were used across all piglets, litters and NEs to ensure the smell of the marker did not have varying effects on behaviour. In each litter, six mix-sexed focal piglets were randomly selected for the whole study; totalling 36 focal piglets per NE. During the three minute focal samples all focal piglets from each litter were observed and any play behaviours were tallied and any targets (object, piglet or sow) recorded. Some miscellaneous behaviours (e.g. 'active fighting' and neighbouring pen contact) were also recorded. For the focal sampling, each focal piglet was observed for a total of 243 minutes pre-weaning.

## 2.4 Phase 2: Comparisons of Post-weaning Play Behaviours

In order to quantify the intensity and duration of antagonistic interactions after weaning, each piglet (N = 117) was lesion scored prior to litter mixing, by counting the number of lesions on each side of the piglet in three sections (head, mid and rump to determine fighting and bullying respectively – Turner et al., 2006; Baumgartner, 2007). These lesion scores acted as baseline lesion scores before entering the weaner pens. Lesion scoring occurred again at 3 days and at 7 days post-weaning.

All weaner pens were continuously digitally video recorded for 24 hours from when the two litters per pen were mixed at weaning. Latency of when first play behaviours from each play behaviour category were recorded, as well as latency for first 'active fighting' (i.e. damaging fighting) to occur. Target piglets and objects were also recorded.

Following the initial 24 hours, each pen was focal sampled using the same methodology as phase 1 as well as the same focal piglets (6 per litter / 12 per weaner pen). Each focal piglet was observed for a total of 270 minutes post-weaning.

### 2.5 Phase 3: Cognitive Tests

One of each sex was randomly selected from each litter to undergo the cognitive test phase, totalling 24 piglets (12 per NE). Consideration of different cognitive tests available resulted in the selection of the Spontaneous Object Recognition Test (Gieling et al., 2011). For one week prior to testing, test piglets were habituated to the hold pen and test pen several times, initially in pairs but later in isolation.

For this study, piglets from the two NEs were compared on object recognition abilities after two different retention times (15 minutes and 60 minutes). For testing, selected piglets from one pen were herded into the hold pen. The hold pen (pen size = 4.5m<sup>2</sup>) contained scattered saw-dust over a solid concrete floor, two handfuls of straw and a mechanical drinker. The piglets were then left for 15 minutes to settle. The order of piglet and pen testing was systematically randomised via Latin Square Design so that day, test order, pen and sex effects were minimised across NEs. The retention time order

involved all piglets from one NE being tested for the 15 minute retention time first and then being tested for the 60 minute retention time.

For stage 1, a piglet was herded into the empty test pen (pen size = 4.5m<sup>2</sup>, solid concrete floors and solid 1.5m high walls). Once the access gate was closed, two identical objects (blue square drinkers; approx. H25cm x L30cm x W27cm) were simultaneously lowered into the pen; one centred on the right wall and the other centred on the left wall. The piglet was exposed to the objects for 5 minutes, all digitally video recorded, so that live observations were not necessary and did not interfere with the test subjects. At the start, the piglet's location in the pen (pen quarter A, B, C or D), and head orientation (left/right) were recorded as well as which object it touched first and the latency to do so. During the five minutes, the piglet's time spent in each quarter was recorded as well as the time spent physically interacting with either object. At the end of the test, the objects were removed and the piglet was returned to the hold pen, where it remained for a specified retention time (15 or 60 minutes) before being retested following the same methods and recordings for stage 1. The only difference being that one of the objects had been replaced by a novel object (a small red/white traffic cone; approx. H35cm x L18cm x W18cm). The side of the pen in which the novel object was placed was systematically randomised by time of day, pen, litter and sex.

## 2.6 Statistical Application

For focal sampling the behaviour tallies for the three minute focal samples were totalled per sample day and for pre-weaning and post-weaning as a whole. For lesion score data, body sections were added together to generate totals for 0, 3 and 7 days post mixing for each piglet and then differences between

0-3 days and 3-7 days were calculated. Statistical comparisons were conducted through linear mixed models (LLM) using the residual maximum likelihood method in Genstat (11<sup>th</sup> Edition). Litter was used as the random factor (encompassing all individual piglet and litter variation). Fixed effects included NE, piglet age, weaning weight, sex, number in litter, NE neighbour, litter order, foster piglet, number in pen, and pen. Statistical significance of terms in the LLMs was tested using the F statistic and  $P < 0.05$ . Any data that were shown to have skewed distribution were transformed by logbase10. Spearman's rank correlations were performed on all behaviour totals pre- and post-weaning in order to establish any significant relationships and their patterns.

Phase 3 data were converted into percentages of time interacting with objects and latencies calculated. Differences were calculated between percentage time interacting with objects for each trial and test phase for the two retention test times. Linear mixed models were conducted using Litter as the random effect and the fixed effects included NE, sex, age, pen, novel object, and novel object side. Spearman's rank correlations were performed for percentage differences of object interactions for the two retention times.

Spearman's rank correlation matrices were used to establish any relationships and patterns between experimental phases.

### 3. Results

#### 3.1 Phase 1: Comparisons of pre-weaning play behaviours

### 3.1.1 Initial four days post-farrowing

During the initial four days post farrowing NEP piglets were significantly quicker to perform first locomotor play than NEC piglets (mean latencies: NEP =  $430.0 \pm 82.9$  mins vs. NEC =  $745.8 \pm 78.8$  mins;  $F = 7.62_{(1,11)}$ ,  $P = 0.020$ ). NE had a significant effect on latency of first piglet social interaction ( $F = 23.61_{(1,11)}$ ,  $P < 0.001$ ), with piglets in NEP ( $84.2 \pm 24.7$  mins) interacting earlier with each other than piglets in NEC ( $246.7 \pm 22.5$  mins). NE also had a significant effect on the first sow-piglet interaction ( $F = 5.27_{(1,11)}$ ,  $P = 0.045$ ), with piglets in NEP performing sow-piglet interactions sooner after farrowing than piglets from NEC (mean latency: NEP =  $85.5 \pm 25.8$  mins; NEC =  $305.0 \pm 74.4$  mins). Farrowing length, mean piglet birth weight, number born and sow parity had no significant effects on latencies for first locomotor play behaviour and social interactions.

A Spearman's correlation matrix highlighted significantly strong positive correlations between performance latency of first locomotor play behaviours and social ( $r_s = 0.719$ ,  $P = 0.008$ ) and sow-piglet ( $r_s = 0.619$ ,  $P = 0.032$ ) interactions.

### 3.1.2 Pre-weaning focal sampling

NE had a tendency to affect total play behaviours pre-weaning, with NEP piglets on average performing more play behaviours than NEC piglets (Table 2). Sex ( $F = 13.92_{(1,71)}$ ,  $P < 0.001$ ) and piglet age ( $F = 5.49_{(1,71)}$ ,  $P = 0.044$ ) had significant effects on total play behaviours pre-weaning. Older piglets on average performed fewer play behaviours than younger piglets and females on average performed more play behaviours than males.

NE showed a significant effect for total pre-weaning locomotor play behaviours; with NEP piglets on average performing higher totals than NEC (Table 2). Sex also had a significant effect ( $F = 12.08_{(1,71)}$ ,  $P = 0.011$ ); with females performing higher totals than males irrespective of NE, although for NEC piglets the difference was small. Foster piglets in NEC tended to have higher mean totals compared to non-fosters, while the opposite was shown in NEP, but these differences were not significant. Weaning weight had no significant effect on total pre-weaning locomotor play behaviours in the LLM analysis, however it did have a significant relationship, shown by a negative correlation ( $r_s = -0.25$ ,  $P = 0.032$ ).

For object play totals NE had no significant effect (Table 2), however sex was highly significant ( $F = 16.17_{(1,71)}$ ,  $P < 0.001$ ), with females performing more object play behaviours than males in both NEs. Foster status did not have a significant effect.

NE had no significant effect on social play behaviours pre-weaning (Table 2). Weaning weight ( $F = 5.57_{(42,71)}$ ,  $P < 0.001$ ) had a highly significant effect, with heavier piglets performing less social play behaviours than lighter piglets ( $r_s = -0.44$ ,  $P < 0.001$ ). Foster status also had a highly significant effect ( $F = 27.58_{(1,71)}$ ,  $P < 0.001$ ), with fosters averaging less social play behaviours than non-fosters.

NE had a highly significant effect on total sow play behaviours pre-weaning (Table 2); with NEP piglets interacting more with their mothers than NEC. Foster status also had a highly significant effect ( $F = 8.89_{(1,71)}$ ,  $P = 0.004$ ), with foster piglets interacting considerably less with the sow than non-fosters irrespective of NE.

NE ( $F = 4.38_{(1,71)}$ ,  $P = 0.063$ ) and sex ( $F = 3.42_{(1,71)}$ ,  $P = 0.069$ ) both tended to affect active fighting behaviour totals pre-weaning. NEP piglets on average fought more than NEC piglets, and females fought more than males (Table 2). Total play invitations were significantly affected by NE; with NEP piglets performing more than NEC piglets (Table 2). There was also a highly significant positive correlation between totals of invitations and social play ( $r_s = 0.40$ ,  $P < 0.001$ ). However, no significant effect for NE was shown for play rejections (Table 2), despite active fighting being significantly positively correlated with play rejections ( $r_s = 0.28$ ,  $P = 0.017$ ).

## 3.2 Phase 2: Comparisons of post-weaning play behaviours

### 3.2.1 Post-weaning initial 24 hour

Data analysis showed no significant difference between NEs for the latency of first play behaviours in the weaner pens ( $F = 1.31_{(1,11)}$ ,  $P = 0.285$ ): NEP =  $9.17 \pm 3.40$  mins vs. NEC =  $6.50 \pm 1.23$  mins. Pen, number per pen, litter mixing order and average weaning weight were all shown to have no effect on latencies for first play behaviours.

Analysis of latency to first fight per pen showed that NE had no effect ( $F = 1.29_{(1,11)}$ ,  $P = 0.288$ ), but the number within each pen did ( $F = 8.69_{(1,11)}$ ,  $P = 0.018$ ), with the pen with fewer individuals showing longer latency before first fighting behaviours occurred. Litters which were neighbours pre-weaning showed a tendency to fight more quickly after mixing compared to non-neighbours ( $F = 5.17_{(1,11)}$ ,  $P = 0.053$ ). All NEP litters only fought non littermates for the first fights after mixing, while NEC showed less preference. Spearman's rank correlations showed that latency for first fight was not significantly correlated with first play behaviours ( $r_s = -0.128$ ,  $P = 0.285$ ).



306

## 307 3.2.2 Lesion scoring

308 Lesion score data showed a significant difference between NEs for the amount of lesions counted  
 309 between 0-3 days ( $F = 5.73_{(1,116)}$ ,  $P = 0.038$ ) and 3-7 days ( $F = 27.05_{(1,116)}$ ,  $P < 0.001$ ) post-weaning. At 3  
 310 days post-weaning NEP piglets had a higher mean lesion score difference compared to NEC piglets,  
 311 however, at 7 days NEC piglets showed little change in lesion scores, while NEP piglets showed a sharp  
 312 decrease in their mean lesion scores (Figure 2).

313

## 314 3.2.3 Post-weaning focal sampling

315 NE was shown to have no significant effect on total play behaviours post-weaning (Table 3). Both sex ( $F$   
 316  $= 28.7_{(1,71)}$ ,  $P < 0.001$ ) and weaning weight ( $F = 34.58_{(42,71)}$ ,  $P < 0.001$ ) were shown to have highly  
 317 significant effects; with males performing more play behaviours than females, irrespective of NE and  
 318 correlational analysis showing that heavier piglets performed less play behaviours than lighter piglets ( $r_s$   
 319  $= -0.46$ ,  $P < 0.001$ ).

320

321 NE also had no effect on all total individual play behaviour categories post-weaning (Table 3): locomotor  
 322 play behaviours, object play and social play. However, Figure 3 demonstrates that total play behaviours  
 323 continue to linearly increase from pre- to post-weaning, despite a temporary reduction as a result of  
 324 weaning (between sample days 9 and 10).

325

Total active fighting behaviours and total play invitations were not significantly affected by NE (Table 3). NE had no effect on total play rejections (Table 3), however weaning weight did have a significant effect ( $F = 2.82_{(42,71)}$ ,  $P = 0.046$ ), with heavier piglets performing less play rejections than lighter piglets.

### 3.3 Phase 3 – Spontaneous Object Recognition Tests

Results for the trial stages of the tests (both objects identical) showed that NE had no significant effect on the percentage time interacting with either object or latencies to approach them.

#### 3.3.1 15-minute retention time

NE had a significant effect on the latency to touch the novel object in the 15 minute retention test ( $F = 9.56_{(1,23)}$ ,  $P = 0.012$ ), with NEP piglets approaching the object more quickly compared to NEC piglets (NEC =  $94.0 \pm 14.1$  secs, NEP =  $43.7 \pm 6.8$  secs). Sex ( $F = 1.03_{(1,23)}$ ,  $P = 0.359$ ) and novel object side (left or right wall) ( $F = 0.00_{(1,23)}$ ,  $P = 0.996$ ) were both shown to have no significant effect on latency to touch the novel object.

NE was shown to have a significant effect on percentage time interacting with the novel object with NEP pigs showing higher percentage time interacting with the novel object compared to the familiar object ( $F = 5.39_{(1,23)}$ ,  $P = 0.045$ ) (Figure 4). Sex also had an effect with males showing a higher percentage time interacting with the novel object in both tests, but it was only significant for the 15 minute test ( $F = 5.32_{(1,123)}$ ,  $P = 0.043$ ).

### 3.3.2. 60-minute retention time

NE had no significant effect on the latency to touch the novel objects ( $F = 0.54_{(1,23)}$ ,  $P = 0.477$ ) or the percentage time interacting with the novel objects ( $F = 0.87_{(1,23)}$ ,  $P = 0.373$ ). Both sex and novel object side had no significant effects on latency or percentage time interacting with the novel object.

### 3.3.3 Relationships across experimental phases

Pre- and post-weaning total play behaviours did not significantly correlate with cognitive abilities in phase 3 (higher interactions times with novel object than familiar object) for either retention times. Interestingly pre-weaning sow-piglet play behaviours showed non-significant tendencies for positive correlations with percentage difference interaction times (objects 1 and 2) for both retention times (15 mins test:  $r_s = 0.40$ ,  $P = 0.054$ , 60 mins test:  $r_s = 0.46$ ,  $P = 0.024$ ).

## 4. Discussion

Overall NE was shown to influence pre-weaning but not post-weaning play behaviours, therefore conservatively supporting the hypothesis that piglets reared in more complex NEs will play more than piglets reared in less complex NEs. NEP piglets developed play behaviours earlier and showed a larger repertoire of play behaviours pre-weaning than NEC piglets, indicating that the effects of NE on play behaviour are short-term and are highly dependent on present environmental stimulus (e.g. more straw, larger space). Previous research supports these findings by demonstrating that piglets reared in more complex (enriched) environments perform more play and exploratory behaviours, being generally more active than piglets reared in less complex (non-enriched) environments (Bolhuis et al., 2005;

Chaloupková et al., 2007; De Jonge et al., 1996; Oostindjer et al., 2011; Petersen et al., 1995; Weary et al., 2002).

Links between play and exploration have also been demonstrated in several studies (e.g. Wood-Gush and Vestergaard, 1991), thus it can be argued that a more complex (stimulating) environment encourages greater exploration and eventual play within it and supports Špinka et al.'s (2001) theory of training for the unexpected. Whilst Špinka et al.'s work supports the current study's findings pre-weaning, once weaned there were no differences in play between the two treatment groups. Post-weaning piglets were in identical environments, and all experienced the same stimulation and this is likely the reason for no difference in play behaviours at this stage.

Pre-weaning NEP piglets may have been experiencing better welfare than NEC piglets if play behaviour frequency is used as an indication of positive welfare; animals only play if they are in a 'relaxed state' and as a result experience positive emotional states (Boissy et al., 2007; Burghardt, 2005; Špinka et al., 2001; Manteuffel et al., 2009).

Play behaviours started earlier in the NEP compared to other 'enriched' environments from other studies, with locomotor play behaviours starting at one day old, while Blackshaw (1997) observed these behaviours not starting until 3-5 days old. Blackshaw (1997) also noted object play occurring during this time, however, our study did not observe any object play behaviour during the four days post-farrowing. NEP piglets showed much greater sow play behaviours compared to NEC piglets. Other studies have observed similar results, with piglets reared in less restrictive environments and with greater access to

the sow, showing considerably higher sow play behaviours than piglets which were not (Blackshaw et al., 1997). The sow and the piglets in NEP could interact more easily with each other in the larger and non restrictive environment, compared to the NEC, therefore allowing play behaviours and other social interactions to be performed. Blackshaw's (1997) results also supported our finding that sow interactions occurred at around 1-2 days old and suggested this was fundamental for developing the sow-piglet maternal bond.

The continuing increase in play behaviours during the pre-weaning period (0-4 weeks) has been observed in other studies, although data from the present study do not show a peak between 2-6 weeks old (Bolhuis et al., 2005; Newberry et al., 1988), but instead shows a continued increase until eight weeks. Although there was a sharp decrease on the days when weaning occurred and play behaviours remained lower than pre-weaning levels for the first week post-weaning. Several studies have demonstrated how weaning results in a decrease in play behaviours (e.g. Donaldson et al., 2002). It is suggested that this is due to the event being novel, and involving an abrupt change in environment, including the withdrawal in milk, resulting in piglets being stressed, hungry and suffering from negative emotional states (e.g. fear) (Boissy et al., 2007; Broom, 2008; Jensen and Stangel, 1992). Therefore the motivation to play is hampered and supports the theories of the function of play being a luxury behaviour only performed when an animal is in a 'relaxed state' (Špinka et al., 2001; Burghardt, 2005).

Weaning also involves piglets being mixed into novel groups; therefore motivations for establishing group social hierarchies will become the priority until dominance is resolved. Lesion score data demonstrated a sharp increase in fighting behaviours during this period, with the majority of individuals all participating and receiving a high number of lesions particularly in the first three days. NEP piglets

showed higher aggression levels than NEC piglets; however this aggression was acute and sharply decreased by day seven, while NEC piglets remained aggressive for the entire seven days, even though the total lesions were lower than NEP piglets at three days. This suggests that piglets reared in more complex environments, with basic access to neighbouring litters (through barred windows) may perhaps resolve social hierarchy disputes quicker than piglets reared in litter-isolated and less complex environments. However from these results we can only infer that environmental complexity and/or access to other litters causes this and without measuring stress physiology, for example, we can only assume that pro-longed aggressive encounters displayed in the NEC piglets cause a welfare detriment. Other studies which have taken these measurements have shown that piglets mixed prior to weaning appear to show decreased aggression and stress responses to mixing at weaning (D'Eath, 2005; De Jonge et al., 1996; Parratt et al., 2006; Schaefer et al., 1990). However, resident-intruder tests (D'Eath, 2005) did demonstrate that socialised piglets were more aggressive to intruder piglets and engaged in fighting more quickly than un-socialised piglets (D'Eath, 2005; Kanaan et al., 2008), therefore supporting the present results. Perhaps piglets reared in more complex and sociable environments are able to develop their social and fighting skills earlier than litter-isolated piglets in less complex environments (D'Eath, 2005), thus providing them with the motivation and confidence to tackle hierarchical disputes quickly and efficiently. If the function of play is to train for the unexpected (Špinka et al., 2001), then a NE which provides greater novelty and complexity would allow for greater experience and play development, and perhaps more successfully preparing piglets for novel social and aggressive interactions at weaning.

Results for cognitive abilities across the two NEs showed that NEP piglets were better at discriminating between familiar and novel objects after a retention time of 15 minutes, but there was no difference in

cognitive abilities for NEs after the 60 minute retention time, suggesting that pigs can discriminate between objects. Pigs reared in more complex environments were more adept at object discrimination, but there is a limit to their declarative memory (Bolhuis et al., 2004; Siegford et al., 2008; Winters et al., 2008; Bracke and Spoolder, 2008). Whilst these findings support our hypothesis that piglets reared in more complex and stimulating NEs are able to develop their socio-cognitive abilities further than piglets reared in less complex NEs, the fact that we found no significant correlations between play behaviour (pre- and post-weaning) and interaction durations with novel objects means it cannot be confirmed whether the increase of play behaviour or the more complex NE resulted in better object discrimination post-weaning.

Similar results regarding retention times have been shown by Kornum and colleagues (2007), where pigs demonstrated the ability to discriminate between objects in spontaneous object recognition tests after 10 minute retention times, but no discrimination was shown after one hour or 24 hours. The results for the latencies to approach the novel object showed that NEP piglets for the 15 minute retention test did approach the novel object more quickly, perhaps suggesting a more adept ability to cope with novelty and being less fearful (e.g. less neophobic than NEC piglets). It could be argued that NEP piglets may not be better at object recognition, but merely are less neophobic due to an optimistic cognitive bias (Douglas et al., 2012), although they would have to show some cognitive understanding that one object is novel. Although comparatively Olsson and colleagues (1999) demonstrated that pigs reared in enriched environments were less likely to approach novel objects and even showed avoidance behaviours compared to pigs reared in non-enriched environments, and they suggested that the lack of stimulation and poor social development in a NE may be factors in developing poorer risk assessment abilities. In the current study pigs from both NEs did comparably approach novel objects, but pigs from

the more complex NE showed more exploration and play behaviours perhaps due to extra stimulation they received pre-weaning (Špinka et al., 2001; Bolhuis et al., 2004). Links between more complex play behaviours and cognitive ability have been suggested (Held et al., 2009), therefore piglets reared in NEP who did develop a broader play repertoire pre-weaning, may have developed their cognitive abilities earlier, resulting in greater object play (seen pre- and post-weaning) as well as a better ability to discriminate between objects in the novel object test and the motivation to explore, compared to NEC piglets. However the results did show large individual variation for both NEs, which might be attributed to individual variation in cognitive abilities (e.g. genetic pre-disposition to memory capacity (Gielsing et al., 2011; Kornum et al., 2007) as well as temperament (Lind and Moustgaard, 2005; Spooler et al., 1996; Wemelsfelder et al., 2000).

There is also the issue of interference during the cognitive tests; research by Mendl (1997), demonstrated how pigs' spatial memory was reduced when disturbances (e.g. isolation or novel food source) occurred during retention intervals. During this study, test pigs were held with two pen mates, and were provided with saw dust and straw to reduce stress. However, perhaps the presence of these items and other individuals acted as disturbances during the retention periods, therefore masking cognitive ability in the 60 minute test, where discrimination may have been more subtle.

Irrespective of NE, sex was shown to have substantial effects on both play and socio-cognitive abilities pre- and post-weaning. Pre-weaning, female piglets played more than males, but this was reversed post-weaning, and in the cognitive tests, males appeared better at discriminating than females. Other studies have shown that on the whole, males play more than females (Blackshaw et al., 1997; Houpt, 2005) and studies on maze tasks in pigs showed no significant sex effect (Gielsing et al., 2011; Siegford et



al., 2008). Perhaps the explanation is simply that females show neurological development earlier than males (Short and Balaban, 1994) and that as exploration and play appear to be closely intertwined (Špinka et al., 2001) the reason for the higher interaction with the novel object was not simply discrimination capability, but the motivation to explore and play, which was starting to reduce in females post-weaning. Perhaps the novel object test shows higher play motivations rather than ability to discriminate, although play is heightened by the presence of novel stimuli (Fagen, 1981; Wood-Gush and Vestergaard, 1991).

#### 4.1 Conclusion

These findings have an impact on the way we house commercial pigs and their related welfare, particularly during neonatal development. This study has suggested that piglets reared in enriched and complex NEs develop greater socio-cognitive abilities which have long term direct and indirect effects on their welfare e.g. reduced chronic aggression post-weaning, reduced stress and increased positive emotional states as the result of play. The study also suggests that the pig's memory and cognitive abilities although great, do have limits which perhaps should be considered in management practices (e.g. fostering and mixing) in order to minimise stress and encourage good welfare.

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Figure Captions

Figure 1: Un-scaled diagrams of the two neonatal environments: a) standard farrowing crate; b) PigSAFE pen with the approximate dimensions. In NEP, the swing door provides an enclosed nest area, but when opened (after 7 days), changes and opens up the environment.

Figure 2: Comparison of mean ( $\pm$ SE) lesion score differences (post weaning) for the two neonatal environments.

Figure 3: Play behaviours from pre-weaning-sample days 1-9 (4-27 days of age) to post-weaning-sample days 10-19 (28-56 days of age). Weaning occurred between sample days 9 and 10.

Figure 4: Mean ( $\pm$ SE) percentage differences for interaction durations with objects in spontaneous object recognition trials (objects identical) and tests (introduction of a novel object) for 15 minute retention time between trial and test for pigs from both neonatal environment treatments: farrowing crate (NEC) and PigSAFE pen (NEP).

Table 1: Ethogram for piglet behaviours.

Play Category	Behaviour	Description
Locomotor/ Individual	Scamper	Two or more forward directed hops in quick succession of each other usually associated with excitability.
	Pivot	Twirling of body on the horizontal plane by a minimum of 90° usually associated with jumping on the spot.
	Toss head	Energetic movements of head and neck in quick succession, in both horizontal and vertical planes.
	Flop	Focal animal drops to the pen floor from a normal upright position to a sitting or lying position. There is no contact with an object or another individual (piglet or sow) which could cause the change in position.
	Hop	Focal animal has either its two front feet or all four feet off the pen floor at one time, through an energetic upwards jumping movement. The animal continues facing the same original direction for the whole of the behaviour.
	Rolling	Lying on back, while rocking entire body in side to side movements. Behaviour is terminated when focal animal returns to an upright position.

Gambolling Energetic running in forward motions within the pen environment.

Normally associated with using large areas of the pen, and occasionally coming into marginal contact with other piglets (e.g. nudge).

Social	Pushing	Focal animal drives its head, neck or shoulders with minimal or moderate force into another piglet's body. Occasionally the behaviour results in the displacement of the target piglet.
	Nudging	Snout of focal piglet is used to gently touch another piglet's body, not including naso-naso contact. Usually occurs in bouts of behaviours in quick succession.
	Chase	Focal animal follows the locomotory movement and direction of another piglet vigorously e.g. running after a target piglet which is also running.
	Push-overs	The focal animal uses its head and shoulders to drive a substantial force at a target piglet, resulting in the target to lose balance and fall-over. A fall is identified by the target piglet losing its footing for at least two feet, resulting in its shoulders or hips coming into contact with the floor.
	Sow	Focal piglet uses its feet to elevate itself onto the body of the sow.
	Climbing	A minimum of two feet must be off the floor and on the sow. Any behaviour directed at the sow's udder is ignored, however attempts to clamber above the udder is included, although the majority will be targeted around the sow's head, neck and shoulders.

	Sow nudging	The snout of the focal piglet is used to gently touch the sow's body, not including naso-naso contact. The behaviour normally occurs in bouts, with the single behaviours occurring in quick succession.
Object	Shake object	While holding an item (e.g. straw) in its mouth, the focal animal energetically moves the item from side to side using its neck and head. This behaviour also includes manipulation of items in a similar fashion, which are fixed at one point (e.g. hooked chains). Item must be visible to the observer when being held in the piglet's mouth.
	Carry object	Animal securely holds an item in its mouth, while moving in a forward direction. Item must be visible to the observer when being held in the piglet's mouth.
Miscellaneous	Play Invite	Focal piglet performs locomotor or social play behaviours, which are directed through face-to-face body orientation to another non-playing piglet. The behaviours are often repeated rapidly and highly energetic.
	Play Reject	Focal piglet which is a target of play invite behaviours from another piglet, responds by turning its head and body a minimum of 90° away from the 'inviting' piglet, and does not reciprocate any play behaviours.
	Active fighting	Focal piglet strikes or bites another piglet with significant force or attempts to do so (e.g. head/shoulder knocks). Normally performed with aggressive vocalisations.

Out of sight Piglet was or has gone out of sight during the 3 minute focal sample. The majority of these incidences occurred when the focal piglet moved into the creep areas, as this was the only major blind spot in the digital recordings.

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Table 2: Means and standard errors (SE) for pre-weaning behaviour totals for NEs and their statistical comparisons. Degrees of freedom = 1,71 for all measures.

Pre-weaning behaviour totals	NEC		NEP		F statistic	P value
	Mean	SE	Mean	SE		
Play Behaviours	150.11	4.14	170.33	5.80	5.06	0.051
Locomotor play	56.81	2.33	68.75	3.12	7.71	0.020
Object play	16.25	1.05	17.92	1.37	1.36	0.268
Sow-piglet play	11.72	1.12	26.89	1.26	33.16	<0.001
Social (piglet- piglet) play	65.33	2.75	56.78	3.29	0.67	0.433
Active fighting	4.64	0.38	6.08	0.40	4.38	0.063
Play invitations	4.25	0.35	7.25	0.54	27.08	<0.001
Play rejections	4.61	0.35	4.89	0.38	0.20	0.661

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Table 3: Means and standard errors (SE) for post-weaning behaviour totals for NEs and their statistical comparisons. Degrees of freedom = 1,71 for all measures.

Post-weaning behaviour totals	NEC		NEP		F statistic	P value
	Mean	SE	Mean	SE		
Play Behaviours	281.19	9.30	294.25	6.16	1.65	0.232
Locomotor play	128.39	6.52	129.25	5.17	0.23	0.645
Object play	52.72	2.20	54.33	2.25	0.24	0.634
Social (piglet- piglet) play	100.08	4.26	110.67	3.80	1.6	0.237
Active fighting	20.72	0.91	18.31	1.20	0.78	0.397
Play invitations	14.86	0.98	14.42	0.87	0.31	0.588
Play rejections	9.33	0.90	6.69	0.54	1.48	0.249

659

660

Figure 1

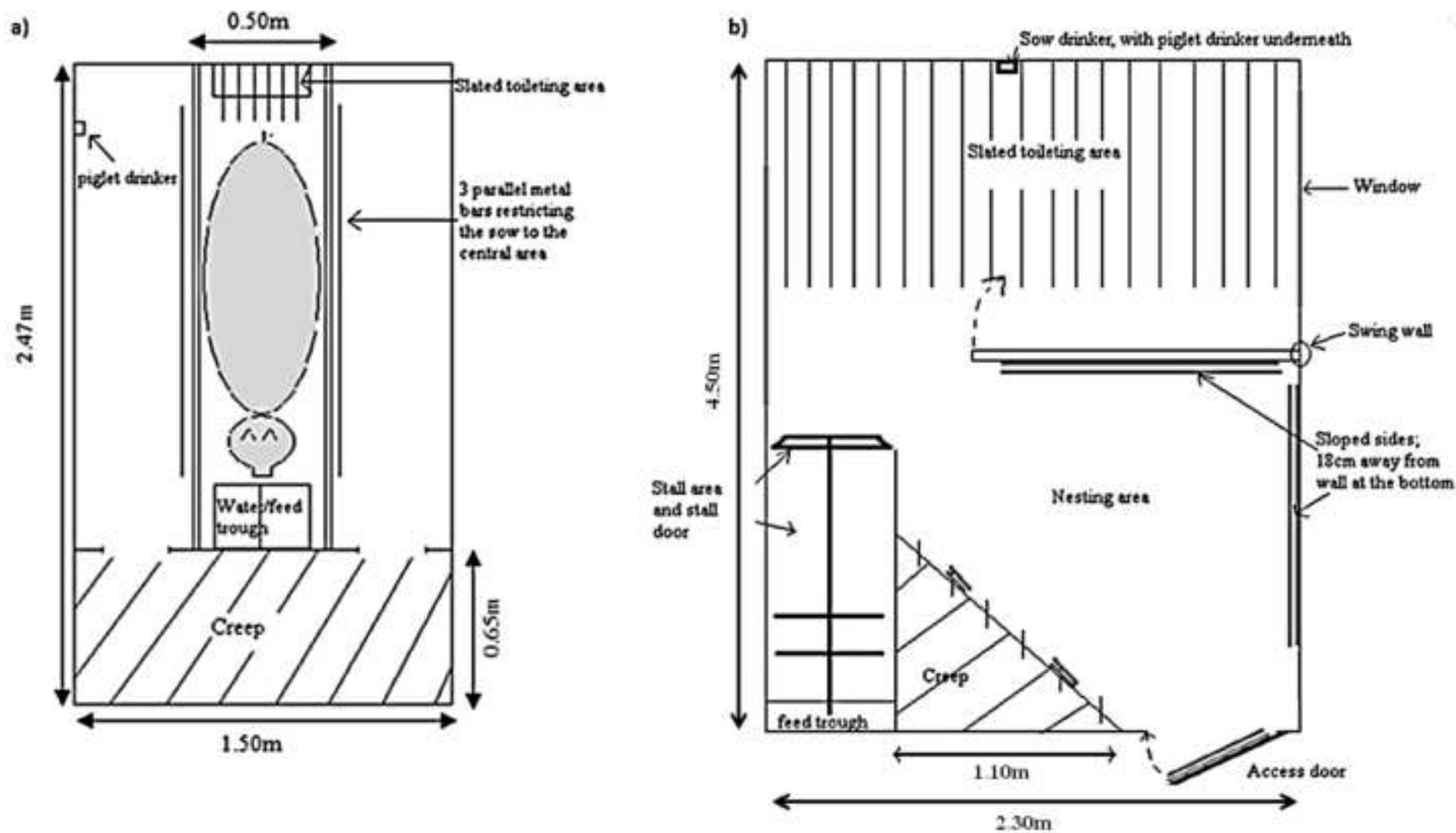




Figure 2

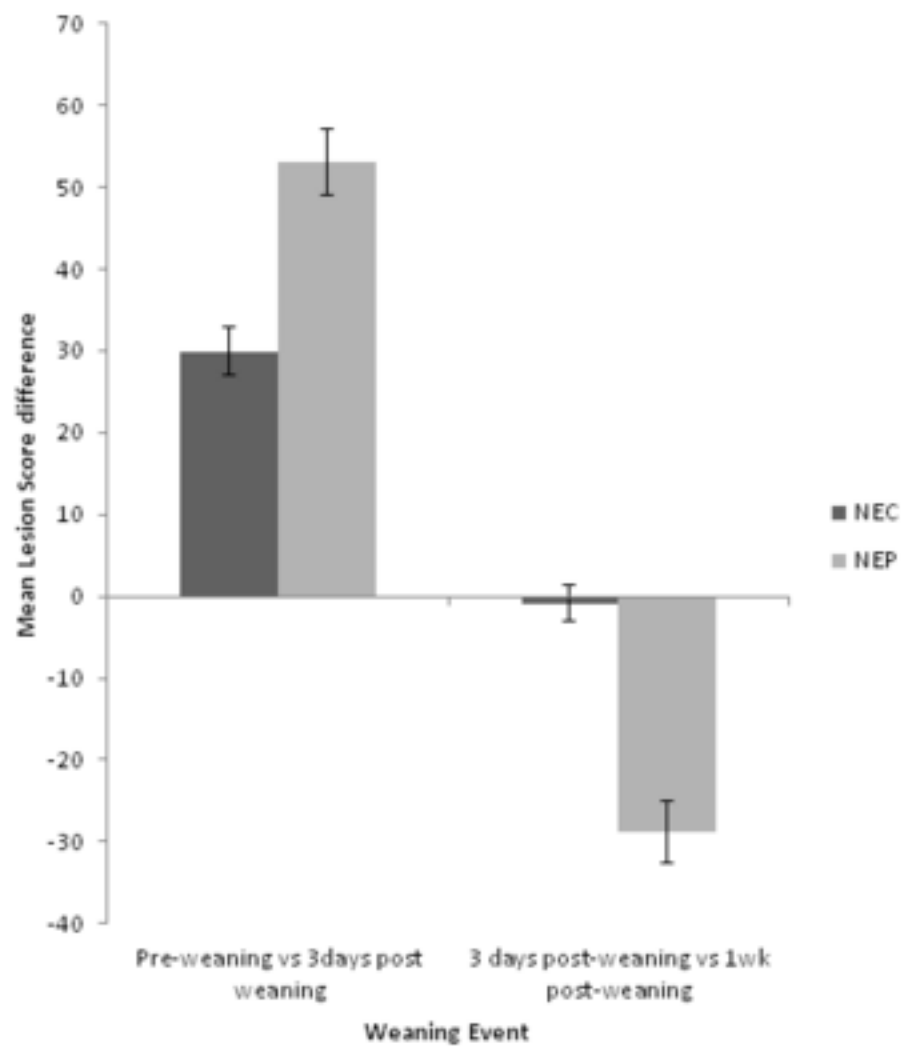


Figure 3

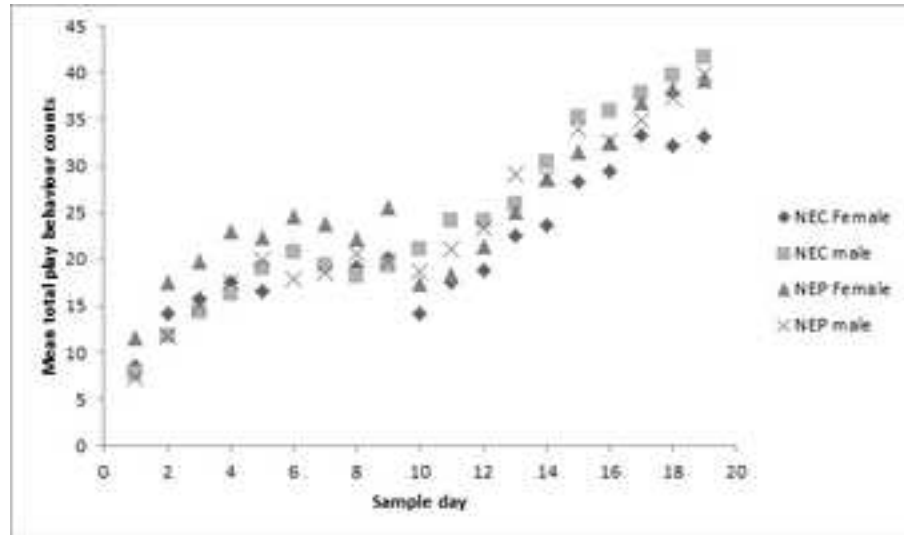


Figure 4

